RESEARCH REPORT
SWINE HEALTH

Title: Infectivity of swine manure from pits at varying lengths of time post infection with Porcine Epidemic Diarrhea (PED) virus - **NPB #14-246**

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Industry Summary:
This project was designed to test manure from deep pits in 30 barns across southern Minnesota and Northern Iowa for the presence of viable PEDV in an effort to understand the risk of transmitting this virus during pit pumping season of 2014. Of the pits we tested, we found that 2 still had infectious virus 4 months after PEDV positive pigs were on the site. This continues to emphasize the need for proper biosecurity measures and planning during the pit pumping season of 2014.

In this study, we sampled 30 manure pits from barns in southern Minnesota and northern Iowa. At each site, manure was sampled through pump outs, using a 10 foot section of PVC piping angled into the pit as far under the animal space as the collection personnel could reach. At each site, a minimum of 3 pump out were sampled. The manure was pooled, and tested for PEDV at the University of Minnesota Veterinary Diagnostic lab by polymerase chain reaction (PCR). Positive samples were then tested by swine bio-assay for presence of live virus. Additionally, samples were sent to Minnesota Valley Testing Laboratory (New Ulm, MN) for complete nutrient profile.

We found that on average, pits had a PCR cycle time value of about 30, indicating a relatively high amount of viral genetic material. When tested by swine bio assay, 2 barns 4 months after having PEDV positive pigs were positive. In the swine bio-assay, 20 mL of manure from one site was administered via a stomach tube to a single pig and was observed for 3.5 days. At the end of the study the pig was taken to the diagnostic laboratory and infection was confirmed by the diagnosticians there.

When we compared the nutrient analysis between sites that had live PEDV and those that did not have live PEDV, we found that those with live PEDV had lower pH (around 7.4 versus greater than 8.0), and higher levels of copper (around 50mg/kg versus less than 40 mg/kg). While it is not clear yet how these two factors influence whether or not a pit has live PEDV, ongoing studies will attempt to describe this.

It is important to note, that it is possible for to us have missed live PEDV in some of the pits. This is because we sampled a small amount of manure from the perimeter of the pit through pump outs.

In conclusion, it appears that some barns will still have live PEDV in them during the pit pumping season of 2014. It is recommended to be very careful about sequencing of equipment when possible and practice good bio security measures. It is hopeful that careful planning and good communication may help minimize the spread of PEDV during this time. Additional studies will be conducted to better understand the risk of manure coming from sow barns where the infection patterns are likely to be very different.

Keywords: PEDV, bio-assay, manure, pumping, pH

Abstract:

Since introduction into the US swine herd, PEDV has spread rapidly across swine producing regions causing dramatic production losses. As the fall of 2014 approaches and manure pit pumping begins enforce, there was growing concern regarding the risk of transmitting viable virus from previously infected sites. Therefore this study was designed to test 30 manure pits for the presence of live PEDV using swine bio-assay. In this study, 0 of 15 sites were positive for live virus at 6 months post infection whereas 2 of 15 sites were positive for live virus 4 months post infection. Pits with live virus had significantly lower pH levels when compared to pits with no live virus. These observations reinforce the importance of biosecurity, especially with pit pumping crews where it is strongly advised to pump negative sites first, and work toward positive sites while paying particular care with those sites most recently infected with PEDV.

Introduction:

Porcine epidemic diarrhea virus (PEDV) was first diagnosed in the United States (US) swine herd in May 20131. Since then it has spread rapidly, and near by the end of June 2013, the virus had infected nearly 50% of 739 participating sow herds in the Swine Health Monitoring Project2. Early case reports from 4 farms included dramatic accounts of 90 – 95% mortality in suckling pigs characterized by profuse watery diarrhea containing undigested milk, vomiting, and dehydration1. Due to the rapid dissemination of this virus in the US swine herd, immediate investigations into the roles of transportation3, aerosol transmission4,5 and feed6 were started quickly. Each study concluded the potential for transmission by its respective route. Epidemiologic investigations into the risk factors of infection and patterns of dissemination have also been conducted and reported in the March14th, 2014 issue of the weekly Swine Health Monitor report2 (Accessed on 9/18/14 at http://www.cvm.umn.edu/sdec/SwineDiseases/pedv/index.htm). This report concluded production type, size, density, onsite rendering pick up and wildlife vectors increased the odds of becoming infected with PEDV. More recently, a spatio-temporal analysis of two PEDV outbreaks in North Carolina and Iowa found significant relationships between the observed number of cases, as well as the distance and time between these cases in both study areas7.

While early studies have shown PEDV to be stable in fecal material and slurry for varying amounts of time from 14 days to more than 1 month, depending on temperature (Goyal #13-215: University of Minnesota Environmental stability of PED (porcine epidemic diarrhea) virus. National Pork Board), little is known beyond that. There is growing concern that the pumping and transportation of swine manure and subsequent application to agricultural fields may be a significant risk factor for transmission of viable PEDV in the swine industry during the fall of 2014.

Objectives:

The objective of this study was to sample manure from wean to finish farms that were either 6 months or 4 months post-PEDV infection and test for the presence of viable virus by swine bio-assay.

Materials and Methods:

Sample site selection – Wean to finish farms were selected during a two week time period in late August through early September 2014 based on documented history of PEDV infected pigs on site. Routes by which PEDV positive pigs arrived at the site included both vertically transmitted (PEDV positive pigs coming from a PEDV positive sow flow) and laterally transmitted (anything other than vertical, ie. contaminated feed, transport, personnel movement, aerosol, etc). Therefore, sites that had PEDV positive pigs in late February or early March of 2014 were selected for the 6 month post-PEDV group and farms that had positive pigs in late April or early May of 2014 were selected for the 4 month post-PEDV group. A survey was completed at each site which ascertained information regarding pit volume, average site inventory, water source, feed and water medications used, washing and disinfecting practices, use of pit additive(s), and presence of crusting or foaming in the pit.

Sample collection – Samples were collected using a 10 foot section of one-half inch, schedule 40, Polyvinyl Chloride (PVC) piping. At each site, samples were drawn from pit pumping ports around the perimeter of each barn. Collections were made from a minimum of 3 to a maximum of 6 pumping ports, distributed evenly around the perimeter of the barn. If barns had less than or equal to 4 accessible pumping ports, each one was sampled. Pumping port covers were removed far enough to allow the passage of the PVC pipe into the manure slurry in the pit while angling the pipe as far underneath the animal space as possible. When collection personnel had extended their reach as far under the animal space as possible, the open end of the PVC pipe was covered with the thumb, the pipe was withdrawn from the pumping port, and the sample was allowed to drain into a bucket by removing the thumb from the end of the PVC pipe. 3 samples were drawn in this manner from each pumping port at different angles under the animal space. Individual pumping port samples from each sample site were pooled into a bucket, mixed, and aliquots were collected. New, clean PVC piping and buckets were used at each sampling site. Samples were stored under refrigeration for no more than 2 days before use, and were frozen at -20 degrees Celsius if storage longer than 2 days was required.

Sample Assays – Aliquots of each sample were sent to the University of Minnesota Veterinary Diagnostic Laboratory for PEDV Polymerase Chain Reaction (PCR) testing. Additionally, a sample of the manure was sent to Minnesota Valley Testing Laboratories in New Ulm, MN for complete nutrient analyses (series 3) with the pH add on.

Swine bio-assay – Samples that tested positive for PEDV by PCR, were then tested by swine bio-assay. 33, three week old barrows from a PRRS, and PED negative sow flow were obtained for this study. At arrival, pigs were administered 0.25ml of Baytril (Bayer Animal Health, Whippany, New Jersey, USA) via sub cutaneous injection using a 1.0 ml tuberculin syringe and a 20 gauge x ¼ inch needle. Rectal swabs were collected using a Stuart's Media collection swab (Becton, Dickenson and Company, Franklin Lakes, New Jersey, USA) and frozen at -20 degrees Celsius. Pigs were housed individually, bedded with pine shavings and fed a standard pelleted nursery ration without products of swine origin ad libitum. Pigs were randomly assigned to a sample site for which they were to be inoculated with. A 20 ml sample of undiluted manure slurry was administered to the pig via a 14 French gastric tube/urinary catheter (Bard Medical, Covington, GA, USA) attached to a 60cc syringe. Pigs were manually restrained, and the tube was slowly passed into the back of the oral cavity allowing the pig to swallow the tube. To ensure the tube had not been inadvertently passed into the trachea, negative pressure was applied to the syringe. If air passed into the syringe, the tube was removed from the mouth of the pig, and the process was started again. If no air was drawn back, the sample was slowly administered to the pig over 5-10 seconds. The tube was pinched closed and withdrawn slowly to prevent regurgitation and the pig was returned to the individual pen. Pigs were observed for a period of 3.5 days and then transported to the University of Minnesota Veterinary Diagnostic Laboratory for necropsy, intestinal PCR, histopathology and Immunohistochemistry.

Four negative controls were used during the study where pigs were inoculated with manure from sites confirmed to be PEDV negative. Two positive controls were conducted where pigs were inoculated with known PEDV positive intestinal

homogenate obtained from a sow farm using it to expose and acclimatize newly arrived replacement gilts to PEDV present on that farm.

Statistics – Descriptive statistics and comparisons using student's two sample t-test using pooled or statterthwaite methods when appropriate were completed using Staistix 9 (Tallahassee, FL, USA).

Results:

Bio-assay

A total of 30 sites were conveniently selected and sampled during the study, of which 15 were from sites that were 6 months post-PEDV (hereafter referred to as 6 month sites) and 15 were from sites that were 4 months post-PEDV (hereafter referred to as 4 month sites). Among the 6 month sites, 14 tested positive by PCR whereas 13 tested positive by PCR among the 4 month sites. Mean PCR cycle time was not significantly different between the 6 month sites (30.2, 95% CI = 28.7, 31.7) and the 4 month sites (29.0, 95% CI = 28.3, 29.7) ($p = 0.1444$).

Of the 14 PCR positive 6 month sites, 0 tested positive for live PEDV by swine bio-assay. Of the 13 PCR positive 4 month sites, 2 tested positive for live PEDV by swine bio-assay. Of the two bio-assay positive samples, the average PCR cycle time value on intestines of inoculated pigs was 15.38. Histopathology from these pigs showed mild villus tip necrosis in 4 of 6 sections and also tested positive by PEDV immunohistochemistry. Clinical signs were not observed in either of these pigs.

All pre-inoculation rectal swabs tested negative by PEDV PCR. All 4 negative control pigs tested negative by PEDV PCR on intestinal tissues at the end of the trials. Both positive control pigs tested positive by PEDV PCR on intestinal tissues. Average cycle time value on positive control pigs was 16.0. Histopathology showed mild atrophic enteritis on 3 to 5 sections, and also tested positive by PEDV immunohistochemistry. Clinical signs were not observed in either of these pigs.

Manure profile analysis

6 month vs 4 month sites –

There were no significant differences in mean pH values between the 6 month sites (8.05, 95% CI = 7.87, 8.22) and the mean pH value of the 4 month sites (7.90, 95% CI = 7.74, 8.06) ($p = 0.1942$). Copper values differed significantly between 6 month sites (mean = 16.29 mg/kg, (95% CI = 11.88, 20.70) and 4 month sites (mean = 47.63 mg/kg, 95% = 41.48, 53.79) ($p < 0.0001$).

Bio-assay positive vs bio-assay negative sites –

When the bio-assay positive sites were compared to all sites in the project, there were no significant differences between mean pH values of bio assay positive sites (pH = 7.25) and bio-assay negative sites (pH = 8.00) ($p = 0.1881$). Significant differences in pH were detected, however, when bio-assay positive sites (pH = 7.25) were compared to only the other bio-assay negative sites sampled 4 months post-PEDV (pH = 7.99) ($p < 0.0001$). Due to limited number of positive sites (n =2), confidence intervals were not able to be calculated.

When the bio-assay positive sites were compared to all sites in the project, there were significant differences between mean copper values of bio assay positive sites (53.7 mg/kg) and bio-assay negative sites (29.4 mg/kg) ($p = 0.0004$). Significant differences in mean copper values were not detected, however, when bio assay positive sites (53.7 mg/kg) were compared to only the other bio-assay negative sites sampled 4 months post-PEDV (46 mg/kg) ($p < 0.0001$). Due to limited number of positive sites (n =2), confidence intervals were not able to be calculated.

No significant differences were detected for any other manure profile parameter measured, or from any factor captured by the site survey, for either comparison.

Discussion:

The results presented here represent the first known report to test viability of PEDV in field samples of manure. There are important limitations to this study which should be acknowledged. First, only 20 mL of manure were tested by bio-assay in a pit that may have contained 0.5 to more than 1 million gallons of liquid manure. Additionally, samples were collected from the perimeter of the barn through pit pump outs. These limited samples may not accurately reflect the true status of the manure from these sites. That said, these data are promising, suggesting sites that were 6 months post-PEDV do not appear to contain large quantities of live virus, however, some sites that were 4 months post-PEDV may yet contain live virus capable of causing infection. These data are corroborated by two accidental exposures of large numbers of young susceptible animals to manure on commercial production sites that would have been classified 6 months post-PEDV during the time of this study. In both instances, the manure tested positive for PEDV by PCR, yet pigs remained clinically and diagnostically negative for PEDV.

These data also suggest a potential link between pH level and viable PEDV. Caution should be used, however, when interpreting this result because of a small number of positive sites (n =2). With this in mind, these data seem plausible given the numerous anecdotal observations that treating barn surfaces with a basic (of a high pH) solution of hydrated lime or “white wash”, seems to be an effective means of killing or reducing the quantity of viable PEDV during the cleaning phase after an outbreak occurs on a site.

Additionally, caution should be used when interpreting differences measured in copper levels as this data is likely confounded by the diets used by the production company (some companies use higher levels of copper for longer periods of time in their diets) contracting pigs in these barns. That said, these data were intriguing enough to report, and additional studies may be needed to elucidate potential mechanisms between copper levels, pH, and viable virus.

Ultimately these data help reinforce the development of previously established protocols for pumping swine manure. Generally, it is advised that crews first pump sites that are free from various diseases and work their way toward sites that are endemically infected. These data might also suggest pumping sites that have been most recently infected with PEDV last. Additionally, proper hygiene is always recommended between sites, including washing and disinfecting vehicles, tools and equipment. If additional days of down-time between sites are practical, it is recommended. Finally, communication with neighboring sites is critical to ensure all efforts have been explored to minimize risk of moving potentially infectious manure around naïve or negative sites.